

Leptomeningeal metastases in patients with human epidermal growth factor receptor 2 positive breast cancer: real-world data from a multicentric European cohort

Ivica Ratoso, Nika Dobnikar, Michele Bottosso, Maria Vittoria Dieci, William Jacot, Stéphane Pouderoux, Domen Ribnikar, Léa Sinoquet, Valentina Guarneri, Tanja Znidaric, Amélie Darlix, Gaia Griguolo

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1. Supplementary Materials and Methods

Identification of breast cancer subtypes

Routine diagnostic pathology reports were used to assess the oestrogen receptor (ER), progesterone receptor (PR), and Human epidermal growth receptor 2 (HER2) status of primary breast cancer tumours or metastatic disease at the time of diagnosis and were evaluated according to then valid American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommendations. Hormone receptor–positive disease was classified as ER+ and/or PR+ disease with a cut off value of 1 %. The methods for detecting ER, PR, and HER2 varied between years 2005 and 2020.

The detection methods for ER or PR

For the detection of **ER**, immunohistochemistry (IHC) methods were as follows:

- **Years 2005 – 2011:** Antibody clone SP1 (Estrogen Receptor, RM-9101-S, 1:25, NeoMarkers ThermoFisher Scientific, Kalamazoo MI), staining platform Autostainer 480 (LabVision™, Denmark), detection DAKO REAL™ EnVision™ Detection System (K5007, RTU, DAKO, Golstrup, DK) or SP1 (790-4325, Ventana Medical System, Tucson AZ, pre-diluted) or Clone 6F11 (Novocastra™).
- **Years 2011 – 2014:** antibody clone SP1 (Estrogen Receptor, RM-9101-S, 1:25, NeoMarkers ThermoFisher Scientific, Kalamazoo MI), staining platform Benchmark XT (Ventana medical system, Tuscon, AZ), detection UltraView universal DAB Detection Kit (760-500, RTU, Ventana medical system, Tuscon, AZ) or 6F11 (PA0153, Leica Biosystems Newcastle, Newcastle UK, pre-diluted) or Clone EP1 (DAKO, diluted).
- **Years 2014 – 2020:** Antibody clone SP1 (Estrogen Receptor, RM-9101-S, 1:200, NeoMarkers ThermoFisher Scientific, Kalamazoo MI), staining platform Benchmark Ultra or Benchmark XT (both Ventana medical systems, Tuscon, AZ), detection OptiView DAB IHC Detection Kit (760-700, RTU, Ventana medical systems, Tuscon, AZ) or 6F11 (PA0153, Leica Biosystems Newcastle, Newcastle UK, pre-diluted) or Clone EP1 (DAKO, Golstrup, DK, diluted).

For the detection of **PR**, IHC methods were as follows:

- **Years 2005 – 2011:** Antibody clone PgR636 (Progesterone receptor, M3569, 1:50, DAKO, Golstrup DK), staining platform Autostainer 480 (LabVision™, Denmark), detection DAKO REAL™ EnVision™ Detection System (K5007, RTU, DAKO, Golstrup, DK) or 1E2 (790-4296, Ventana Medical System, Tucson AZ, pre-diluted).
- **Years 2011 – 2013:** antibody clone 1E2 (anti-PR 1E2 Rabbit Monoclonal primary Antibody, 790-2223, RTU, Ventana medical systems, Tuscon, AZ), staining platform Benchmark XT (Ventana medical systems, Tuscon, AZ), detection UltraView universal DAB Detection Kit (760-500, RTU, Ventana medical systems, Tuscon, AZ) or 16 (PA0322, Leica Biosystems Newcastle, Newcastle UK, 1:100).
- **Since 2014:** Antibody clone 16 (Novocastra™ Liquid Mouse Monoclonal Antibody Progesterone Receptor, NCL-L-PGR-312, 1:800, Leica Biosystems, NewCastle, UK), staining

platform Autostainer 480 (LabVision™, Denmark), detection EnVision™ FLEX High pH (K8000, RTU, DAKO, Golstrup, DK) or 16 (PA0322, Leica Biosystems Newcastle, Newcastle UK, 1:100) or Clone PgR636 (DAKO, Golstrup, DK, diluted).

The detection of HER2 status

By IHC:

- **Years 2005 – 2010:** Polyclonal antibody (HercepTest™ for Techmate™ Instruments, K5206, RTU, DAKO, Golstrup, DK), staining platform Autostainer 480 (LabVision™, Denmark), detection DAKO REAL™ EnVision™ (K5007, RTU, DAKO, Golstrup, DK).
- **Years 2011 – 2020:** Antibody clone 4B5 (VENTANA anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody; 790-2991, RTU, Ventana medical systems, Tuscon, AZ), staining platform Benchmark XT (Ventana medical systems, Tuscon, AZ), detection UltraView universal DAB Detection Kit (760-500; RTU, Ventana medical systems, Tuscon, AZ) or antibody A0485 (DAKO, Golstrup, DK).

By fluorescent in situ hybridization (FISH):

- The detection of HER2 status by FISH was performed in all cases regardless of IHC score and the same method was used in **years 2005 – 2020:** Path Vysion HER2 DNA probe kit II (06N46-036, RTU, Abbott Vysis, Abbott Park, IL).

2. Supplementary tables

Supplemental table 1: Systemic treatment received for metastatic breast cancer before LM diagnosis.

Treatment modality	All patients (n=82, 100%)	HR+ (n=49, 100%)	HR- (n=33, 100%)
Endocrine therapy			
0	53 (64.6)	22 (44.9)	33 (100)
1 line	23 (28.0)	21 (42.9)	0
≥2 lines	6 (7.3)	5 (12.2)	0
Chemotherapy			
0	25 (30.5)	16 (32.7)	9 (10.9)
1 line	25 (30.5)	14 (28.6)	11 (13.4)
≥2 lines	32 (39.0)	18 (21.9)	13 (15.6)
Any anti-HER2 therapy*			
0	17 (23)	11 (13.4)	6 (7.3)
1 line	35 (47.3)	21 (25.6)	14 (17.1)
≥2 lines	22 (19.9)	11 (13.4)	11 (13.4)
Missing data	8 (9.8)		

* Anti-HER2 therapy includes trastuzumab, trastuzumab emtansine, lapatinib.

Abbreviations: n = Number; LM = Leptomeningeal metastases; HR = Hormonal receptors.